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以细菌中烯醇式丙酮酸转移酶为作用靶点的新天然抑制剂*

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摘要：肽聚糖是细菌细胞壁的重要成分，烯醇式丙酮酸转移酶（EPT）是调节肽聚糖合成初始阶段的关键酶。通过活性筛选，发现了三个对 EPT 有抑制活性的天然产物（化合物 **1**～**3**），它们皆为 EPT 的新抑制剂。体外抗真菌实验发现，化合物 **2** 和 **3** 对光滑念珠菌具有较好的生长抑制作用。另外，化合物 **3** 还具有一定的肿瘤细胞毒活性。

关键词：烯醇式丙酮酸转移酶；天然抑制剂；细胞壁；抗菌
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New Natural Inhibitors Targeting Bacterial Enolpyruvyl Transferase*

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Abstract: Bacterial enolpyruvyl transferase (EPT) is one of the key enzymes acting in the initial stage of peptidoglycan synthesis of bacterial cell walls. Through inhibition assay，three new natural inhibitors (**1-3**) were found against EPT. Inhibitor **2** and **3** showed antifungal activities against fungus *Candida glabrata*，and inhibitor **3** showed cytotoxicities against several cancer cells.

Key words: Enolpyruvyl transferase；Natural inhibitor；Cell wall；Antibacterial

Bacterial cells possess a cell wall，while mycoplasma or mammalian cells don't possess it. Peptidoglycan is the major structural component of bacterial cell walls. Inhibition of its biosynthesis will prevent cell wall formation and lead to cell death eventually. Thus，peptidoglycan is an important target of many clinically used antibiotics，such as penicillin，β-lactams，imipenems，cephalosporins and glycopeptides (Yuan *et al.*，2007).

Peptidoglycan is a heteropolymer comprised of linear chains of polysaccharides containing *N*-acetylmuramic acid and *N*-acetylglucosamine，cross-linked by short peptidic chains (Lortal and Chapot-Chartier，2005). Many enzymes involved in peptidoglycan biosynthesis，such as N-acetyl-glucosamine-1-phosphate uridyltransferase (GlmU) (Sulzenbacher *et al.*，2001)，UDP-N-acetyl-glucosamine enolpyruvyl transferase (EPT or MurA) (Bachelier *et al.*，2006)，UDP-N-acetyl-

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enolpyruvyl glucosamine reductase (MurB) (Benson *et al.*, 1996), penicillin-binding proteins (PBPs) (Tuomanen, 1986) and D-glutamic acid-adding enzyme (MurD) (Štrancar *et al.*, 2006) etc . EPT (EC 2.5.1.7) is responsible for the first step in the cytoplasmatic biosynthesis of peptidoglycan precursor molecules . It catalyzes the transfer of phosphoenolpyruvate (PEP) to the 3-hydroxyl group of UDP-N-acetylglucosamine (UDPAG) yielding enolpyruvyl-UDP-N-acetylglucosamine and inorganic phosphate (Bachelier *et al.*, 2006) . For its potential pharmaceutical interest in antibacterial agents, EPT was an attractive target for antibiotic discovery . Many EPT specific inhibitors were found, such as fosfomycin, derivatives of 5-sulfonoxy-anthranilic acid, pyrazolopyrimidine and cyclic disulfide (Baum *et al.*, 2001; Eschenburg *et al.*, 2005), and some of them showed good inhibitory activities against bacteria .

In our previous research for natural antibacterial inhibitors, some extracts from plants were found with potent inhibitory activities against EPT (Jiang *et al.*, 2003) . In this paper, three new natural inhibitors (**1 - 3**) of EPT were found with IC_{50} s below 50 μg/mL (Fig . 1) through EPT assay . The antifungal results indicated that compounds **2** and **3** showed inhibitory activities against fungus *Candida glabrata* (CG) (Table 1) .

Materials and Methods

Compounds **1 - 3** (Fig . 1) were isolated by us with purities > 95% . Detailed purifications and identifications were described before (Zhou *et al.*, 2005; Stermitz and Castro, 1983; Mu and Li, 1982) . Fosfomycin (Sigma, P5396) is used as a reference compound for EPT assay .

Inhibitory activity against EPT was performed as described previously (Baum *et al.*, 2001; Eschenburg *et al.*, 2005) . Compounds were diluted with reaction buffer (pH 7.4, 12.5 μmol/L Tris-HCl, 5% BSA-Tris) in the presence of 1 μg/mL *Enterobacter cloacae* EPT solution (provided by Bayer AG (Germany)) . Then the reaction was initiated by the addition of 125 μmol/L UDPAG (Sigma, U-4375) and 32.5 μmol/L PEP (Fluka, 79415) . After 2 h functional time at 37℃, indicator containing 0.045% malachite green base and 3.16% ammonium molybdate tetrahydrate was added, and the OD values at 630 nm were measured in a plate reader (Molecular Devices, Spectra MAX340) . IC_{50} s of compounds against fungus CG were determined by Yeast Nitrogen Glucose (YNG) method . Test compounds were diluted with the YNG medium (14.5 mg/mL $Na_2HPO_4 \cdot 12H_2O$, 3.55 mg/mL KH_2PO_4, 6.7 mg/mL yeast nitrogen base dehydrated, 10 mg/mL glucose) . Inocula containing 1.3×10^3 cells/mL of CG in log phase growth were prepared in YNG cultures . After incubated for 55 h at 37℃, the OD values were monitored at 540 nm in a plate reader (Molecular Devices, spectra MAX340) .

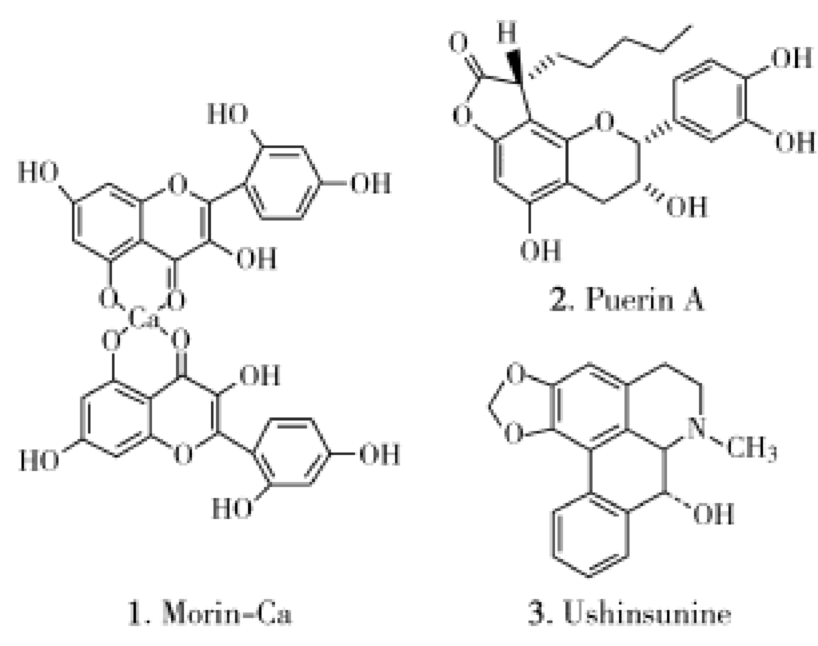

Fig . 1 Chemical structures of natural inhibitors **1 - 3**

Results and Discussion

The increasing number of antibiotic-resistant bacteria has fuelled interest in the development of new antibiotics and other antibacterial agents . EPT, one of the important enzymes of peptidoglycan biosynthesis of bacterial cell walls, is a potential target for antibacterial agents (Lanzetta *et al.*, 1979) . Through inhibition assay, three new natural inhibitors of EPT were found with chemotypes of flavone (**1**), flavane (**2**) and alkaloid (**3**) (Fig . 1) . These three inhibitors have no apparently structural similarity to fosfomycin . Moreover, the antifungal tests also showed that two inhibitors of them (**2** and **3**) are with potent inhibitory activities against fungus CG (Table 1), which indicated that EPT inhibition might be one of their antibacterial

Table 1 Inhibitory activities of inhibitors **1 - 3** against bacterial EPT and fungus CG

Comp .	Plant sources	IC_{50} (μg/mL)	
		EPT	CG-YNG
1	*Artocarpus pithecoqallus*	33.29	NA
2	*Camellia sinensis* var. *assamica*	41.35	2.88
3	*Michelia yunnanensis*	40.47	3.90

NA, Not Active

mechanisms. Additionally, compound **1-3** were tested for their cytotoxicities against cancer cells by sulfurhodamine B assay (Skehan *et al.*, 1990). Results indicated that compound **3** showed cytotoxicities against cancer cell line A549 (lung cancer), BGC-823 (gastric cancer cell line) and MDA-MB-231 (breast cancer cell line) with IC_{50} s below 10 μg/mL.

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